

WHAT IS CLAIMED IS:

1. An attenuated strain of a bacteria, said bacteria comprising altered DNA adenine methylase (Dam) activity such that the bacteria are attenuated.
2. The attenuated strain of Claim 1, wherein the altered activity reduces Dam activity.
3. The attenuated strain of Claim 2, wherein the altered activity eliminates Dam activity.
4. The attenuated strain of Claim 1, wherein the altered activity is obtained by a deletion in a *dam* gene.
5. The attenuated strain of Claim 1, wherein the altered activity is obtained by an increase in expression of Dam.
6. The attenuated strain of Claim 1, wherein the bacteria is an attenuated form of *Haemophilus*.
7. The attenuated strain of Claim 1, wherein the bacteria are an attenuated form of a bacteria selected from the group consisting of: *Salmonella enterica* serovars, *E. coli*, Non Typable *Haemophilus influenza*, *Streptococcus pneumoniae*, *Helicobacter pylori*, *Shigella* Spp., *Vibro cholerae*, *Yersinia* Spp., *Neisseria meningitidis*, *Porphyromonas gingivalis*, and *Legionella pneumonophila*.
8. The attenuated strain of Claim 1, wherein the bacteria are an attenuated form of a bacteria selected from the group consisting of *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus somnus*, *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, and *Mannheimia haemolytica*.
9. The attenuated strain of Claim 1, wherein the altered activity is obtained by an artificially engineered change in a genome of a wild-type pathogenic bacteria.
10. The attenuated strain of Claim 9, wherein the change in the bacteria's genome is a change selected from the group consisting of a deletion, an insertion and a mutation of the native sequence.
11. The attenuated strain of Claim 1, wherein the altered activity is obtained by a heterologous nucleotide inserted into a wild-type pathogenic bacteria.

12. The attenuated strain of Claim 11, wherein the heterologous nucleotide is operatively inserted into a plasmid and expresses DNA adenine methylase.

13. The attenuated strain of Claim 1, wherein the bacteria are an attenuated form of a pathogenic bacteria selected from the group consisting of *Escherichia*, *Vibrio*,
5 *Yersinia* and *Salmonella*.

14. The attenuated strain of Claim 13, wherein the bacteria are an attenuated form of a pathogenic salmonella bacteria selected from the group consisting of *S. typhimurium*, *S. enteritidis*, *S. typhi*, *S. abortus-ovi*, *S. abortus-equi*, *S. dublin*, *S. gallinarum*, and *S. pullorum*.

10 15. The attenuated strain of Claim 13, wherein the bacteria are an attenuated form of *E. coli*.

16. The attenuated strain of Claim 13, wherein the bacteria are an attenuated form of *V. cholerae*.

15 17. The attenuated strain of Claim 13, wherein the bacteria are an attenuated form of *Y. pseudotuberculosis*.

18. The attenuated strain of Claim 1, wherein the bacteria are an attenuated form of a bacteria selected from the group consisting of *Shigella*, *Haemophilus*, *Bordetella*, *Neisseria*, *Pasteurella* and *Tremonema*.

19. The attenuated strain of Claim 1, wherein the bacteria are an attenuated
20 form of a bacteria selected from the group consisting of *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus somnus*, *Actinobacillus pleuropneumoniae*, *Pasterurella multocida*, and *Mannheimia haemolytica*.

20. The attenuated strain of Claim 1, wherein the bacteria are an attenuated form of *Haemophilus*.

25 21. A composition comprising: a pharmaceutically acceptable excipient; and bacteria with altered DNA adenine methylase (Dam) activity which altered DNA adenine methylase activity renders the bacteria non-pathogenic.

22. The composition of Claim 21, further comprising an adjuvant.

23. An immunogenic composition comprising: a pharmaceutically acceptable
30 excipient; and live bacteria comprising altered DNA adenine methylase activity wherein

the altered activity reduces virulence relative to the bacteria with wild-type Dam activity.

24. The immunogenic composition of Claim 23, wherein the Dam activity is altered by a heterologous nucleotide.

5 25. The immunogenic composition of Claim 23, wherein the Dam activity is altered by a mutation in the bacteria's genome which mutation alters a gene involved in expressing Dam in a manner selected from the group consisting of reduced expression, no expression, overexpression expression of a form of Dam altered from Dam native to the bacteria.

10 26. A method comprising steps of: administering to a subject capable of generating an immune response a composition comprising a pharmaceutically acceptable excipient, an immunogenic dose of altered bacteria with altered DNA adenine methylase (Dam) activity which bacteria are attenuated; and allowing the composition to remain in the subject for a time and under conditions to allow the
15 subject to generate an immune response to the bacteria and produce antibodies specific to the bacteria.

27. The method of Claim 26, wherein the antibodies generated are IgG type antibodies.

20 28. The method of Claim 27, wherein the IgG antibodies are highly specific for an antigen of the bacteria.

29. The method of Claim 26, wherein the bacteria remain in the subject under conditions and for a period of time sufficient to allow for B cells of the subject to undergo isotype switching and further for the B cells to undergo clonal expansion.

25 30. The method of Claim 29, wherein an amount of antibodies produced by the subject exceeds 150% of an amount of antibodies which would be produced by the subject administered unaltered bacteria in amount equivalent to the immunogenic dose of altered bacteria.

31. The method of Claim 26, wherein the bacteria are selected from the group consisting of *Escherichia*, *Vibrio*, *Yersinia* and *Salmonella*.

30 32. The method of Claim 26, wherein the bacteria are *Haemophilus*.

33. A method of eliciting an immune response in an individual, comprising:
administering an immunogenic composition to an individual in an amount sufficient to
elicit an immune response wherein the composition comprises a pharmaceutically
acceptable carrier and a bacteria comprising a genome characterized by a mutation
5 altering DNA adenine methylase (Dam) activity such that the bacteria is attenuated;
allowing the composition to remain in the individual for a time and under conditions to
allow the individual to generate an immune response.

34. The method of Claim 33, wherein the bacteria are *Haemophilus*.

35. An attenuated strain of a bacteria, said bacteria comprising a cloned *dam*
10 gene capable of altered DNA adenine methylase (Dam) activity such that said bacteria
are attenuated and suitable for use as a live vaccine.

36. The attenuated strain of Claim 35, wherein said altered activity increases
Dam expression.

37. The attenuated strain of Claim 36, wherein said increased Dam expression
15 is obtained by control of said cloned *dam* gene by a promoter.

38. The attenuated strain of Claim 37, wherein said promoter is selected from
the group consisting of a *lac* promoter, *tac* promoter, *araBAD* promoter, *trc* promoter,
trp promoter, T7, SP6, or T5 bacteriophage promoters, a native promoter from that
species, or other appropriate promoter.

39. The attenuated strain of Claim 35, wherein said bacteria is a species of the
20 *Pasteurellaceae* family.

40. The attenuated strain of Claim 35, wherein the bacteria are an attenuated
form of a bacteria selected from the group consisting of *Pasteurella multocida*,
Mannheimia haemolytica, *Actinobacillus pleuropneumoniae*, *Haemophilus somnus*,
25 *Actinobacillus suis*, and *Haemophilus parasuis*.

41. A composition comprising:

a pharmaceutically acceptable carrier in combination with a bacteria
demonstrating altered DNA adenine methylase (Dam) activity, said altered activity
being overexpression of Dam,

whereby said overexpression of Dam renders the bacteria non-pathogenic and suitable as an attenuated live bacterial vaccine.

42. The composition of Claim 41, wherein said over expression is obtained by control of said cloned *dam* gene by a promoter.

5 43. The composition of Claim 42, wherein said promoter is selected from the group consisting of a *lac* promoter, *tac* promoter, *araBAD* promoter, *trc* promoter, *trp* promoter, T7, SP6, or T5 bacteriophage promoters, a native promoter from that species, or other appropriate promoter.

10 44. The composition of Claim 41, wherein said bacteria is a species of the *Pasteurellaceae* family.

45. The composition of Claim 41, wherein said bacteria are an attenuated form of a bacteria selected from the group consisting of *Pasteurella multocida*, *Mannheimia haemolytica*, *Actinobacillus pleuropneumoniae*, *Haemophilus somnus*, *Actinobacillus suis*, and *Haemophilus parasuis*.

15 46. A method of producing an attenuated live vaccine comprising:
cloning a *dam* gene of a bacterial species into a plasmid;
said plasma comprising a promoter capable of controlling the expression of said *dam* gene;

20 introducing said plasmid to a wild type of said bacteria species so as to produce bacteria which demonstrate altered DNA adenine methylase (Dam) activity such that said bacteria are rendered non-pathogenic and suitable for use as an attenuated live bacterial vaccine.

47. The method of Claim 46, wherein said expression of said *dam* gene is overexpressed.

25 48. The method of Claim 47, wherein said overexpression is obtained by control of said cloned *dam* gene by a promoter.

30 49. The method of Claim 48, wherein said promoter is selected from the group consisting of a *lac* promoter, *tac* promoter, *araBAD* promoter, *trc* promoter, *trp* promoter, T7, SP6, or T5 bacteriophage promoters, a native promoter from that species, or other appropriate promoter.

50. The method of Claim 46, wherein said bacteria is a species of the *Pasteurellaceae* family.

51. The method of Claim 46, wherein the bacteria are an attenuated form of a bacteria selected from the group consisting of *Pasteurella multocida*, *Mannheimia*
5 *haemolytica*, *Actinobacillus pleuropneumoniae*, *Haemophilus somnus*, *Actinobacillus suis*, and *Haemophilus parasuis*.

52. The method of Claim 46, wherein said plasmid is stabilized by treating a mutation in a chromosome of said bacteria, said mutation being lethal to said bacteria under predetermined conditions.

10 53. A method of providing a level of immunity to infection by a pathogenic bacteria comprising:

providing an attenuated strain of bacteria, said attenuated strain comprising a cloned *dam* gene capable of altered DNA adenine methylase (Dam) activity such that said bacteria are attenuated and suitable for use as a live vaccine;

15 providing a pharmaceutically acceptable carrier;

combining said attenuated strain of bacterial with said carrier to produce a dose of vaccine suitable for use by a subject capable of being infected by said bacteria;

administering to said subject said dose of vaccine in sufficient quantity to illicit an immune response to said pathogenic bacteria, said response being sufficient to
20 produce antibodies to said pathogenic bacteria.

54. The method of Claim 53, wherein said altered Dam activity increases Dam expression.

55. The method of Claim 54, wherein said increased Dam expression is obtained by control of said cloned *dam* gene by a promoter.

25 56. The method of Claim 55, wherein said promoter is selected from the group consisting of a *lac* promoter, *tac* promoter, *araBAD* promoter, *trc* promoter, *trp* promoter, T7, SP6, or T5 bacteriophage promoters, a native promoter from that species, or other appropriate promoter.

57. The method of Claim 53, wherein said bacteria is a species of the *Pasteurellaceae* family.
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58. The method of Claim 53, wherein the bacteria are an attenuated form of a bacteria selected from the group consisting of *Pasteurella multocida*, *Mannheimia haemolytica*, *Actinobacillus pleuropneumoniae*, *Haemophilus somnus*, *Actinobacillus suis*, and *Haemophilus parasuis*.

5 59. A method of producing an attenuated live vaccine comprising:
providing a pathogenic bacteria having a *dam* gene and a chromosomal promoter for said *dam* gene;

altering the chromosomal promoter for said *dam* gene,
whereby said altered promoter of said *dam* gene causes altered
10 expression of DNA adenine methylase (Dam) by said pathogenic bacteria such that said pathogenic bacteria are rendered non-pathogenic and suitable for use as an attenuated live bacterial vaccine.

60. The method of Claim 59, wherein said expression of said *dam* gene is overexpressed.

15 61. The method of Claim 59, wherein said altering of said promoter comprises replacement of said promoter.

62. The method of Claim 61, wherein said promoter is selected from the group consisting of a *lac* promoter, *tac* promoter, *araBAD* promoter, *trc* promoter, *trp* promoter, T7, SP6, or T5 bacteriophage promoters, a native promoter from that species,
20 or other appropriate promoter.

63. The method of Claim 59, wherein said bacteria is a species of the *Pasterurellaceae* family.

64. The method of Claim 59, wherein the bacteria are an attenuated form of a bacteria selected from the group consisting of *Pasteurella multocida*, *Mannheimia*
25 *haemolytica*, *Actinobacillus pleuropneumoniae*, *Haemophilus somnus*, *Actinobacillus suis*, and *Haemophilus parasuis*.

65. A method of providing a level of immunity to infection by a pathogenic bacteria comprising:

providing an attenuated strain of bacteria, said attenuated strain comprising a
30 bacteria having a *dam* gene under the control of an altered promoter and being capable

of altered DNA adenine methylase (Dam) activity such that said bacteria are attenuated and suitable for use as a live vaccine;

providing a pharmaceutically acceptable carrier;

combining said attenuated strain of bacterial with said carrier to produce a dose
5 of vaccine suitable for use by a subject capable of being infected by said bacteria;

administering to said subject said dose of vaccine in sufficient quantity to illicit an immune response to said pathogenic bacteria, said response being sufficient to produce antibodies to said pathogenic bacteria.

66. The method of Claim 65, wherein said altered Dam activity increases Dam
10 expression.

67. The method of Claim 65 wherein said altered Dam activity decreases Dam expression.

68. The method of Claim 65, wherein said promoter is selected from the group consisting of a *lac* promoter, *tac* promoter, *araBAD* promoter, *trc* promoter, *trp*
15 promoter, T7, SP6, or T5 bacteriophage promoters, a native promoter from that species, or other appropriate promoter.

69. The method of Claim 65, wherein said bacteria is a species of the *Pasteurellaceae* family.

70. The method of Claim 65, wherein the bacteria are an attenuated form of a
20 bacteria selected from the group consisting of *Pasteurella multocida*, *Mannheimia haemolytica*, *Actinobacillus pleuropneumoniae*, *Haemophilus somnus*, *Actinobacillus suis*, and *Haemophilus parasuis*

71. A method of producing an attenuated live vaccine comprising:
providing a pathogenic bacteria having a native *dam* gene;
25 causing a genetic alteration affecting said *dam* gene,
whereby said alteration of said *dam* gene causes altered expression of DNA adenine methylase (Dam) by said pathogenic bacteria such that said pathogenic bacteria are rendered non-pathogenic and suitable for use as an attenuated live bacterial vaccine.

72. The method of Claim 71, wherein said genetic alteration comprises replacing said native *dam* gene of said pathogenic bacteria with a different *dam* gene.

73. The method of Claim 71, wherein said genetic alteration comprises causing a mutation of said native *dam* gene of said pathogenic bacteria.

5 74. The method of Claim 73, wherein said mutation of said native *dam* gene comprises using a cloned native *dam* gene of said pathogenic bacteria and mutating said native *dam* gene by a method selected from the group consisting of homologous recombination, transposon mutagenesis and site directed mutagenesis.

75. The method of Claim 71, wherein said expression of said *dam* gene is
10 overexpressed.

76. The method of Claim 71, wherein said expression of said *dam* gene is reduced.

77. The method of Claim 71, wherein said bacteria is a species of the *Pasteurellaceae* family.

15 78. The method of Claim 71, wherein the bacteria are an attenuated form of a bacteria selected from the group consisting of *Pasteurella multocida*, *Mannheimia haemolytica*, *Actinobacillus pleuropneumoniae*, *Haemophilus somnus*, *Actinobacillus suis*, and *Haemophilus parasuis*.

79. The method of Claim 71, wherein said genetic alteration affecting said *dam*
20 gene comprises causing a mutation in at least one gene other than said *dam* gene, said at least one gene being upstream or downstream of said *dam* gene and capable of affecting Dam production or activity.

80. The method of Claim 71, wherein said altered Dam expression comprises altered Dam having less activity than Dam expressed by native pathogenic bacteria.

25 81. A method of providing a level of immunity to infection by a pathogenic bacteria comprising:

 providing an attenuated strain of bacteria, said attenuated strain comprising a bacteria having a *dam* gene, said *dam* gene expressing altered DNA adenine methylase (Dam) activity such that said bacteria are attenuated and suitable for use as a live
30 vaccine;

providing a pharmaceutically acceptable carrier;
combining said attenuated strain of bacterial with said carrier to produce a dose
of vaccine suitable for use by a subject capable of being infected by said bacteria;
administering to said subject said dose of vaccine in sufficient quantity to illicit
5 an immune response to said pathogenic bacteria, said response being sufficient to
produce antibodies to said pathogenic bacteria.

82. The method of Claim 81, wherein said altered Dam activity increases Dam
expression.

83. The method of Claim 81 wherein said altered Dam activity decreases Dam
10 expression.

84. The method of Claim 81, wherein said bacteria is a species of the
Pasteurellaceae family.

85. The method of Claim 81, wherein the bacteria are an attenuated form of a
bacteria selected from the group consisting of *Pasteurella multocida*, *Mannheimia*
15 *haemolytica*, *Actinobacillus pleuropneumoniae*, *Haemophilus somnus*, *Actinobacillus*
suis, and *Haemophilus parasuis*.